

1 Luminescent Device

2

3 The present invention relates to a luminescent
4 device comprising a gaseous tritium light source.
5 The device may be used, for example, to calibrate
6 luminometers and other scientific apparatus
7 measuring optical output.

8

9 Different types of scientific apparatus may be used
10 to measure optical readings and frequently find
11 utility in chemistry, biochemistry, biotechnology
12 and medicine. Such optical readings are an
13 effective, reliable and safe method for detection
14 and analysis of molecules and living cell dynamics.
15 Luminometers are one example of such scientific
16 apparatus, and are used to measure the luminous
17 output or luminescence of samples. The luminometer
18 is based on a light-sensitive device termed a
19 photomultiplier.
20

1 Other examples of light measuring equipment include
2 a CCD (Charge Coupled Device) camera based imaging
3 device such as the "Berthold Night Owl", a
4 scintillation counter, photomultiplier, a
5 fluorometer, a spectrophotometer and a photodiode
6 (in particular an avalanche photodiode).

7
8 It is important that apparatus reliant on optical
9 analysis is regularly calibrated to ensure
10 consistency of results. Current optical apparatus
11 calibration devices may comprise a plurality of
12 light emitting diodes of varying intensities. The
13 apparatus is calibrated by checking that the
14 reading of the apparatus corresponds to the known
15 intensity of the light emitted from each of the
16 light emitting diodes. Such calibration is also
17 important when cross-referencing results from
18 different machines.

19
20 These known calibration devices are expensive, and
21 require a power source. This renders them
22 relatively untransportable. The known calibration
23 devices are also bulky and occupy the entire sample
24 space allocated in the apparatus. Thus during
25 calibration of the apparatus, testing must be
26 stopped to insert the calibration device into the
27 apparatus. It is not therefore possible to check
28 the calibration of the machine whilst measuring
29 test samples. There is thus a risk that the
30 accuracy of the apparatus may decrease between
31 calibrations, i.e. during testing, so that test
32 results may be less accurate than is desirable.

1 WO 94/05983 discloses a multi-photomultiplier which
2 utilises a radioactive material to provide a light
3 output. Each photomultiplier component of the
4 multi-photomultiplier described in WO 94/05983 is
5 calibrated against another photomultiplier in the
6 same multi-photomultiplier.

7
8 According to a first aspect of the present
9 invention there is provided a luminescent device
10 comprising a gaseous tritium light source (GTLS)
11 which provides a light output of pre-determinable
12 intensity.

13
14 Tritium (^3H) is a radioactive gas that emits
15 electrons which produce light through scintillation
16 when they collide with a phosphor substance.
17 Tritium has a half-life decay of (12.43 +/- 0.05)
18 years and after this time the activity of the
19 tritium source (and thus its luminescence) is
20 decreased by half. The intensity of the light
21 output will slowly decrease over time in accordance
22 with this half-life decay. As the date of
23 manufacture of the luminescent device is known, the
24 half-life correction may be accurately calculated.
25 The half-life correction may be calculated by means
26 of a computer programme or from a half-life graph.

27
28 Thus, in contrast to WO 94/05983 discussed above,
29 the present invention relates to a device where a
30 gaseous tritium light source provides a light
31 output of predeterminable intensity. The equipment
32 to be tested is compared to a light source of pre-

1 determinable intensity rather than being tested
2 relative to another photomultiplier.

3

4 Preferably a number of distinct devices according
5 to the present invention are provided, each
6 providing a different pre-determinable light
7 intensity. This facility for having a range of
8 different pre-determinable light outputs is
9 especially useful in the calibration of scientific
10 apparatus measuring optical output, for example a
11 luminometer, and enables calibration of the
12 apparatus across the whole required range of light
13 intensity. To achieve reduced light intensity, the
14 device of the invention may comprise a light
15 filtering means which predeterminably alters the
16 intensity of the light output to produce a reduced
17 light output. Suitable light reducing means
18 include a neutral density filter, and the use of
19 differing neutral density filters (e.g. of 1.0
20 giving 10% transmission; 2.0 giving 1%
21 transmission) allowing the luminescence of the
22 device to be reduced by a predetermined amount.
23 Desirably the light outputs are selected to test
24 the accuracy of the apparatus across the whole
25 range of light intensity measurable. Where a
26 luminometer is to be calibrated using one or more
27 devices according to the present invention,
28 preferably the device or devices will test the
29 accuracy of the luminometer from at least 400 to
30 650 nm, suitably from at least 450 to 610 nm.

31

1 The luminescent device is desirably small enough to
2 be housed in a sample holder of the scientific
3 apparatus (e.g. luminometer, fluorometer,
4 spectrophotometer, CCD camera, photodiode (like an
5 avalanche photodiode), photomultiplier,
6 scintillation counter or the like).

7
8 Preferably the luminescent device is shaped and
9 sized to be suitable for insertion into an
10 individual well of a standard size well plate, for
11 example a 96, 384 or 1536 well plate. As the
12 luminescent device of the present invention is
13 small enough to be housed in a single well of a
14 sample holder of a luminometer or other scientific
15 apparatus measuring optical output, it is possible
16 for the luminescent device to be left in the
17 apparatus during use, even when other wells contain
18 test materials.

19
20 The calibration of the scientific apparatus can
21 therefore be checked for accuracy at each instance
22 of use of the luminescent device of the present
23 invention.

24
25 The luminescent device of the present invention may
26 typically comprise the GTLS sealed in a housing
27 which is not easily broken under normal working
28 conditions. Suitably the housing is shatter, heat,
29 cold and moisture resistant. Whilst the housing
30 may be formed of any suitable material, examples
31 include aluminium, brass, steel, plastics (e.g.
32 polypropylene, acrylics and the like), carbon fibre

1 and ceramics. However at least one portion of the
2 inner housing will usually be transparent or
3 translucent (i.e. permits transmission of
4 luminescence) and is unreactive to tritium.
5 Mention may be made of glass (for example sapphire
6 glass), plastic or a combination of these
7 materials. Alternatively, the housing may include
8 an aperture through which the light output is
9 measured. In this embodiment, the GTLS will be
10 retained within the housing by a suitable means,
11 e.g. snug fit of the GTLS within the inner surface
12 or, more usually an adhesive material and generally
13 an outer casing including a transparent or
14 translucent portion will be present.

15
16 Optionally, the housing for the GTLS is itself
17 placed into a chamber of an outer casing having at
18 least one optically transparent or translucent
19 portion to permit transmission of the luminescence
20 from the tritium source. The outer casing
21 facilitates easy handling of the housing which is
22 generally small and also acts as a suitable
23 receptacle for holding any light filter required.
24 The outer casing is typically formed from metal,
25 preferably stainless steel, although other
26 materials (e.g. brass, aluminium, plastics,
27 ceramics etc) can also be used. The transparent or
28 translucent end is suitably formed from glass or
29 plastic. Optionally the transparent or translucent
30 end comprises a neutral density filter.

31

1 The luminescent device may comprise colouring means
2 to alter the colour of the light output to produce
3 a coloured light output.

4

5 Typically the GTLS comprises 10 to 20 mCi of
6 tritium, suitably 15 to 20 mCi, preferably 18 mCi
7 (0.666 GBq) of tritium. A suitable GTLS for use in
8 the present invention is available commercially
9 from mb-microtec ag (Niederwanger, Switzerland).

10

11 In one embodiment the luminescent device according
12 to the invention is sized and shaped to fit within
13 a well in a well plate or the like. In this
14 embodiment, the GTLS will normally be located
15 within an inner housing which itself will be
16 located within an outer casing. For convenience of
17 handling (and especially removal of the device for
18 the well) the outer casing will be of a magnetic
19 material, such as steel. Optionally, the GTLS is
20 located within the inner housing in a snug fit, so
21 that the ends of the GTLS are not able to emit
22 light and this improves the accuracy of the device
23 for calibration or comparative purposes. The GTLS
24 will typically be 4.5 mm x 1.6 mm.

25

26 In an alternative embodiment the GTLS may be fixed
27 within a single housing and an array of filters
28 spaced along the length of the GTLS. Conveniently
29 the filters will be arranged in order of optical
30 density. In this embodiment, the array of filters
31 in a single device facilitates calibration of a
32 microscope or CCD camera, and use of a single light

1 source ensures calibration across the different
2 filters.

3

4 In a further embodiment a scalebar graticule may be
5 etched onto a filter so that the device may be used
6 for measurement, typically of a sample viewed by a
7 microscope or CCD camera. Photolithography may be
8 used to manufacture the scalebar and the scale may
9 be shown in mm or μm depending upon the apparatus.

10

11 According to a further aspect of the present
12 invention there is provided a kit comprising two or
13 more luminescent devices as described above, each
14 providing a light output of pre-determinable and
15 distinct intensity. Thus each of the luminescent
16 devices provides a light output of a different pre-
17 determinable intensity to the other devices present
18 in the kit, and suitably the different intensities
19 provided span the entire range of light intensity
20 measurable by the scientific apparatus.

21

22 Optionally, the kit comprises 3, 4, 5, 6, or more
23 devices, for example may contain 10, 12, 15 or 20
24 devices.

25

26 The kit may also include indicia recording the
27 date(s) of manufacture of the devices, and means to
28 calculate the intensity of the light output at any
29 time from the date(s) of manufacture.

30

31 In some embodiments it may be desirable for the
32 device of the present invention to include a

1 magnetic component. The presence of a magnetic
2 component allows the use of a magnetic handling
3 tool and is especially useful for facilitating
4 removal of small devices of the present invention
5 from wells, such as from the well of a 96 well
6 plate. Conveniently the magnetic component may be
7 provided by use of an outer casing of a magnetic
8 material such as steel.

9
10 The kit may also comprise colouring means to alter
11 the colour of the light output. Suitably the light
12 output of each luminometer calibration device is
13 altered by the colouring means, to a different
14 colour, and the kit provides a range of coloured
15 light outputs.

16
17 Preferably the colouring means comprises one or
18 more phosphors. Suitably the colouring means is
19 provided by a phosphor coating on the GTLS housing.

20
21 According to a further aspect of the present
22 invention there is provided a colourimetric
23 equipment calibration device having a luminescent
24 sample comprising GTLS which provides a light
25 output of pre-determinable intensity and colouring
26 means to alter the colour of the light output to
27 produce a coloured light output.

28
29 According to a further aspect of the present
30 invention there is provided a method of calibrating
31 light measuring apparatus, comprising the steps of;

32

1 placing a luminescent device comprising
2 gaseous tritium light source (GTLS) which
3 provides a light output of pre-determinable
4 intensity in the apparatus; and

5
6 adjusting the reading of light output of the
7 apparatus to the pre-determined intensity of
8 the light output of the luminescent device.

9
10 Where the luminescent device comprises colouring
11 means to alter the colour of the light output to
12 produce a coloured light output, the apparatus
13 tested may be colourimetric equipment.

14
15 According to a further aspect of the present
16 invention there is provided a light measuring
17 apparatus comprising a luminescent calibration
18 device comprising GTLS, wherein the luminescent
19 calibration device is housed in a sample holder of
20 the apparatus.

21
22 According to a further aspect of the present
23 invention there is provided a method of analysing a
24 sample, said method comprising the steps of;
25 i) calibrating an apparatus able to detect light
26 output using a device as described above;
27 ii) inserting said sample into the calibrated
28 apparatus and obtaining a reading therefor.

29
30 The sample may be any suitable sample comprising
31 molecules and/or living cells. Usually the
32 apparatus will be able to quantify the light output

1 reading and may be for example, a luminometer, a
2 fluorometer, a spectrophotometer, a scintillation
3 counter, a photomultiplier, a photodiode (like an
4 avalanche photodiode) or a CCD camera. The method
5 may be applicable for techniques including drug
6 discovery, high throughput screening (especially
7 using a light reporter), molecular biology and
8 diagnostic applications, but other uses are not
9 excluded.

10

11 The present invention will now be described by way
12 of example only with reference to the accompanying
13 drawings in which;

14

15 Figure 1 show a side view of a GLTS insert within
16 an inner housing formed from a material such as
17 aluminium, brass, plastics or the like.

18

19 Figure 2 shows a cross-sectional side view of the
20 inner housing containing the GTLS of Fig.1.

21

22 Figure 3 shows a perspective view of the inner
23 housing of Figs. 1 and 2.

24

25 Figure 4 shows the light output from the device of
26 Figs. 1 to 3.

27

28 Figure 5 is a cross-sectional view of a device
29 according to the invention having the housing of
30 Figs. 1 to 4 located within an outer casing and
31 with a filter located thereon.

32

1 Figure 6 is a cross-sectional view of an outer
2 housing for a device according to the present
3 invention modified for 384 well plates.

4

5 Figure 7 shows a cross-sectional view of a device
6 according to the present invention using the outer
7 casing of Fig. 6.

8

9 Figure 8 shows a cross-sectional view of an outer
10 casing for a device according to the present
11 invention for use in PCR or conical well plates.

12

13 Figure 9 shows a cross-sectional view of a device
14 according to the present invention using the outer
15 casing shown in Fig. 8.

16

17 Figure 10 shows a longitudinal cross-section of a
18 device according to the present invention designed
19 for use in a microscope or CCD camera.

20

21 Figure 11 shows a lateral cross-section of the
22 device of Fig. 10.

23

24 Figure 12 shows a top view of the device of Fig.
25 10.

26

27 Figure 13 shows an exemplary neutral density filter
28 array for use in the device of Figs. 10 to 12.

29

30 Figure 14 shows a longitudinal cross-section of
31 device according to the present invention for use

13

1 in a self-luminescence scale bar or graticule
2 calibration device.

3

4 Figure 15 shows a lateral cross-section of the
5 device according to Fig. 14.

6

7 Figure 16 shows a top view of the device according
8 to Fig. 14.

9

10 Figure 17 shows an exemplary scale bar graticule
11 filter which may be used in the device of Figs. 14
12 to 16.

13

14 Figure 18 shows data from three luminescent devices
15 according to the present invention over a 24 hour
16 period measured using a Mithras LB 940 luminometer
17 (Berthold).

18

19 Figures 19 to 23 illustrate laser etching of
20 luminescent devices according to the present
21 invention.

22

23 Figure 24 shows a longitudinal cross-section of a
24 magnetic handling tool suitable for handling
25 luminescent devices of the present invention.

26

27 Figure 25 shows a lateral cross-section through
28 line A-A in Fig. 24.

29

30 Figure 26 is a photograph of three luminescent
31 devices according to the present invention. Well
32 A1 corresponds to calibration device A of Fig. 18;

1 Well A2 corresponds to device B in Fig. 18 and Well
2 A3 corresponds to the device C in Fig. 18.

3
4 With reference to the Figures, Figures 1 to 5 show
5 an exemplary luminescent device according to the
6 present invention designed for use in 96 well
7 plates. The luminescent device (1) is constructed
8 with an outer casing (6) constructed from stainless
9 steel (416). The outer casing is susceptible to a
10 magnetic field which enables the device to be
11 easily extracted from the 96 well plate using a
12 magnetic handling tool (for example as shown in
13 Figures 24 and 25). The gaseous tritium light
14 source (GSLs) (3) is fixed in place within an inner
15 housing (2) using a silicon based adhesive. An
16 aperture (4) in the top of housing (2) allows light
17 to be admitted (see arrows at Figure 4) and since
18 the aperture is of a given diameter this means that
19 the light output is uniform. The GTLS (3) within
20 the housing (2) as shown in Figures 1 to 4 may be
21 located within the outer casing (6) using an
22 adhesive. A filter (5) formed of glass or other
23 material is then secured across the aperture (4)
24 for example using adhesive. The filter (5) can be
25 of different optical density and exemplary filters
26 include neutral density filters of 1.0 giving 10%
27 transmission, neutral density filter of 2.0 giving
28 1% transmission of neutral density filter of 3.0
29 giving 0.1% transmission. Coloured filters may
30 alternatively be used to filter what light of a
31 specific wavelength.

32

1 An alternative embodiment of the present invention
2 is shown in Figures 6 and 7 and illustrators
3 modified design for the luminescent device for a
4 384 well plate. Figure 6 shows an outer casing (6)
5 which may conveniently be formed of magnetic metal,
6 such as stainless steel. The size of the outer
7 casing will be selected for insertion into an
8 individual well of a 384 well plate but typically
9 the length of the casing shown in Figure 6 would be
10 approximately 9mm. Figure 7 illustrates the formed
11 device with the GTLS 3 being prelocated into a
12 tubular housing (2) which may for example be
13 aluminium. One end of the tubular housing (2)
14 maybe sealed using a suitable sealant, for example
15 silicon glue (8). The opposite end of the inner
16 housing (2) may be sealed with a transparent or
17 translucent material (9) for example glass, such as
18 sapphire glass. A glass filter (5) is placed over
19 the free end of the inner housing such that light
20 is emitted through aperture (7) of the outer casing
21 (6).

22

23 An alternative embodiment of luminescent device
24 according to the present invention is illustrated
25 in Figure 9 and is suitable for use in PCR or
26 conical well plates. An outer housing (6) is shown
27 in Figure 8 and again an inner housing (2) similar
28 to that illustrated in Figures 1 to 4 is present
29 and contains the GTLS (3) a filter (5) is located
30 over the top of the inner housing (2) and light is
31 emitted through apertures (4) and (7).

32

1 Figures 10 to 13 illustrate a luminescent device
2 according to the present invention designed for
3 calibration of a microscope, CCD camera or other
4 imaging system. In this embodiment the GTLS kit
5 (3) is located within an inner housing (2) and is
6 secured therein either through the internal size
7 and shape of the inner housing (2) and/or through
8 the use of an adhesive. A filter (5) is located
9 over the GTLS. An exemplary filter having an array
10 of different neutral densities thereon is
11 illustrated in Figure 13 and demonstrates the
12 option of having different light outputs with a
13 single GTLS lightsources. At each end of the
14 neutral density filter array is a small bar (10 and
15 10') in which the light is not filtered for
16 comparative purposes.

17
18 Figures 14 to 17 illustrate an alternative
19 embodiment of the present invention in which the
20 luminescent device can be used as a self
21 luminescence scale bar or graticule calibration
22 device. The longitudinal cross section, lateral
23 cross section and top view are similar to those of
24 Figures 10, 11 and 12, but Figure 17 shows an
25 alternative exemplary filter in which a scale bar
26 graticule has been etched thereon using lithography
27 or mask techniques (similar to those used during
28 production of a semi-conductor chip) and in which
29 the scale can be selected from millimetres to
30 micrometers.

31

1 Figure 18 shows data from a calibration device over
2 24 hours measured using a Mithras LB 940
3 luminometer (Berthold). Three different devices
4 according to the present invention were measured,
5 each having a different density filter thereon.
6 The devices are labelled A, B and C in the graph.
7 Each device was measured for 0.1 seconds, at 360
8 second intervals over 24 hours. The average
9 intensity of calibration device A was 1011763
10 relative light units (RLU); B equals 99163 RLU and
11 C equals 27326 RLU.

12

13 Figures 19 to 23 illustrate the option of laser
14 etching a luminescent device according to the
15 present invention. Each device is labelled with
16 the product type and with a unique serial number.
17 Such labelling allows the luminescent device to the
18 calibrated manufacture and to trace throughout its
19 lifetime.

20

21 Figures 24 and 25 illustrate an exemplary magnetic
22 handling tool for extracting luminescent devices
23 according to the present invention and having a
24 magnetic component within their manufacture from
25 well plates, for example from 96 or 384 well
26 plates. In the exemplary magnetic handling tool a
27 neodymium disk magnet is fixed into a magnetic rod.
28 Other magnet types could alternatively be used.

29

30 Figure 26 illustrates the devices according to the
31 present invention (the devices as illustrated in
32 Figure 18) in use in a 96 well plate. In sample A1

1 (corresponding to sample A of Figure 18) the light
2 intensity of the GTLS is strong and the GTLS is
3 clearly visible. In sample A2 (corresponding to
4 sample B in Figure 18) a greater degree of
5 filtering has been applied and in sample A3
6 (corresponding to sample C in Figure 18) the
7 filtering has again been increased.